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# **I FLUIDI SUPERCRITICI E LE LORO APPLICAZIONI**

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## DEVELOPMENT OF INDUSTRIALLY-USEFUL SYNTHETIC PROCESSES IN SUPERCRITICAL CARBON DIOXIDE

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### ABSTRACT

It has been previously demonstrated that several types of chemical reactions can be carried out in supercritical fluid media, including those catalyzed by enzymes and active solids. The research reported in this study has focused on the development of industrially-useful processes conducted in high pressure  $\text{CO}_2$ , to convert agriculturally-derived substrates (seed oils) into methyl esters and monoglycerides. An enzyme catalyzed synthesis has been developed using a novel bench-top extraction/reaction system for the methanolysis of soybean and corn oils. Quantitative conversions in excess of 95% have been achieved with this system and fatty acid methyl ester (FAMES) compositions are equivalent to those utilized in biodiesel-based fuels. A variation of this reaction system has also proven useful in the formation of FAMES for nutritional labeling analysis. High pressure glycerolysis has also been studied in  $\text{SC- CO}_2$  with the intent of producing monoglycerides from seed oils and glycerol. The equilibrium-controlled reaction was conducted in a modified high pressure, stirred autoclave over the pressure range of 5-62 MPa and temperatures as high as 250°C. Yields of 45% monoglyceride have been achieved to date in the resultant product mixture.

### 1. INTRODUCTION

The technical advantages associated with extractions performed with supercritical fluid media [1] have also been found to have merit for conducting reactions in the same solvents [2]. When reactions are conducted in a supercritical fluid, such as  $\text{CO}_2$ , one gains the advantage of an environmentally-compatible medium which allow for the benign disposal of the solvent as well as compatibility with many foodstuffs and bioactive materials. In addition to these benefits, supercritical fluids can also allow control over the following variables when conducting a reaction, as noted in Table 1:

Another advantage attendant in initiating reactions in  $\text{SC- CO}_2$  is that the reaction process can be integrated with either an extraction or fractionation process, using the variable density of the supercritical fluid medium, to selectively deliver or remove the reactants or products to the

reaction vessel. This is particularly advantageous in removing unwanted by-products or moieties which might interfere in the progress of the reaction.

Improvement of Mass Transfer  
Control of Reactant/Product Solubility  
Lower Reaction Temperatures  
Regeneration of Catalyst  
Control of Reaction Rate  
Control of Product Distribution

**Table 1:** Advantages to conducting reactions in supercritical fluids.

A number of different types of reactions have been studied under supercritical conditions, although some only by accident or in a cursory fashion. These are listed in Table 2 below.

Type	References
Enzymatic	3,4
Polymerization	5,6
Conversions in SC-H <sub>2</sub> O	7,8
Heterogeneous Catalysis	9
Pyrolytic	10
Photolytic	11
Reactions of Analytical Significance	12

**Table 2:** Types of reactions conducted in supercritical fluids

The reactions reported in this research were designed to yield industrially-useful products that are derivable from natural feedstocks, particularly vegetable oils. They include a lipase catalyzed esterification to produce fatty acid methyl esters (FAMES) of value as synthetic reaction intermediates or as an alternative fuel to diesel (biodiesel) [13]; and a high pressure glycerolysis reaction [14] that has the potential to produce monoglycerides for use as ingredients in food compounding [15].

For the methanolysis reaction, we have performed the reaction with the aid of a relatively new lipase, isolated from *Candida antarctica*, Novozyme 435 immobilized on polyacrylamide beads. The enzyme is a nonspecific lipase, thereby assuring complete conversion of the triglyceride moieties comprising the vegetable oil to their esterified form. The reaction has been performed in a flow system consisting of a micro bench top reactor, in which the reactants and SC-CO<sub>2</sub> are delivered via a series of syringe pumps. This continuous flow system has some advantages with respect to varying the reactant compositions and minimizes the use of large quantities of the enzyme catalyst. In addition to this system, we have also scaled up the transesterification home-built system, incorporating an extraction vessel filled with soy flakes to simulate a combination of a supercritical fluid extraction (SFE) followed in-line by a supercritical fluid

reaction (SFR). A miniaturized variant of this concept has also proven of value in the synthesis of FAMES for nutrition labeling analysis [16].

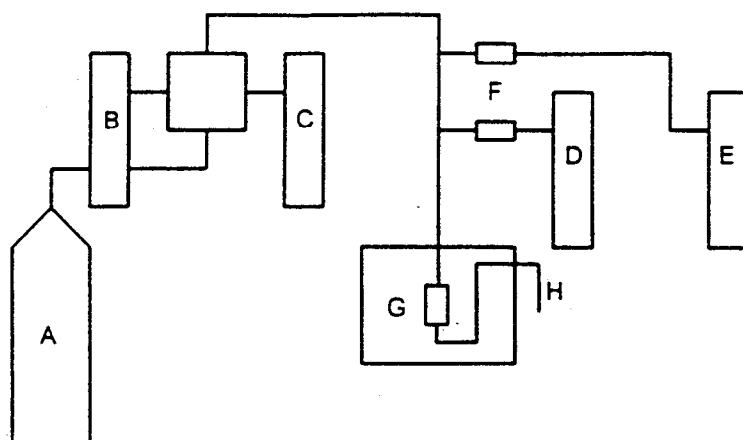
The glycerolysis reaction is based on the previously reported study [17] in which carbon dioxide was reported to catalyze the conversion of vegetable oils to mixtures of monoglycerides and diglycerides. The use of CO<sub>2</sub> at close to ambient conditions offers obvious advantages over metallic salts in catalyzing the above reaction. For this reason, we were curious as to whether CO<sub>2</sub> in its supercritical state would confer any advantages in conducting the above process at lower temperatures, but higher pressures. This reaction was studied with the aid of stirred high pressure autoclave as described in the experimental section below.

## **2. EXPERIMENTAL**

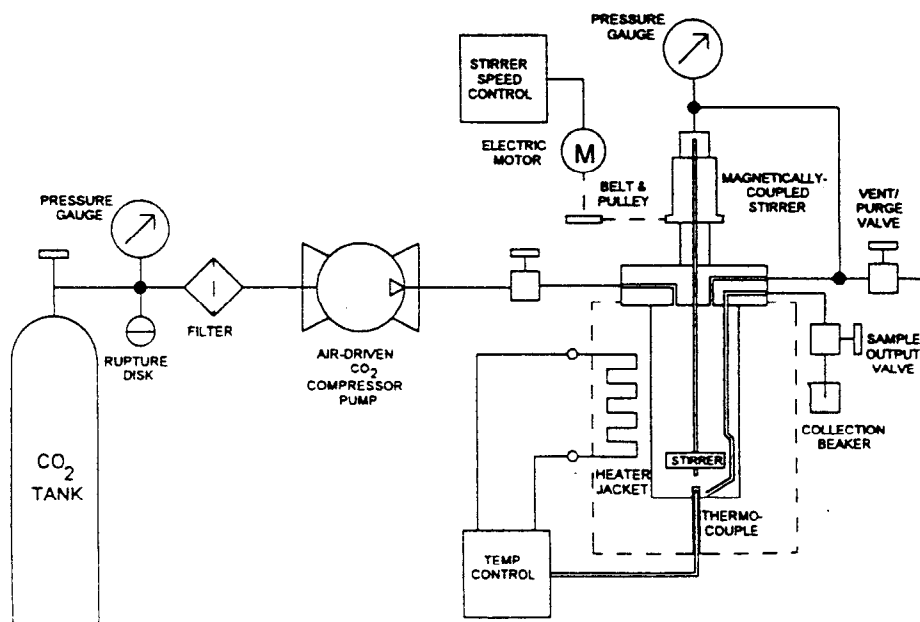
Figure 1 illustrates the continuous flow system developed for studying the methanolysis of vegetable oils. As shown in the schematic, CO<sub>2</sub> is pumped from a cylinder (A) by two syringe pumps (B, C) operating in series, so as to assure a continuous flow of liquefied gas. Methanol for the formation of FAMES is delivered by another syringe pump (E) and enters the flowstream ahead of the introduction of seed (corn) oil. The vegetable oil is pumped by yet another syringe pump (E) and joins the methanol laden CO<sub>2</sub> stream before it enters the reaction vessel held at supercritical conditions. The reaction vessel containing the lipase catalyst is a conventional analytical SFE cell (10 mL) contained in a Isco SFX-2-10 extractor module. Both the extractor and associated syringe pumps were obtained from Isco, Inc. (Lincoln, Nebraska, USA). The conversion of the dissolved seed oil takes place in the cell containing the enzyme catalyst (G) and the product FAMES are collected in a vial inserted after a heated coaxial restrictor (Isco). Typical flow rates for the methanol and CO<sub>2</sub> for this reaction were 4  $\mu$ L/min and 1 mL/min, respectively.

FAME compositions were analyzed by gas chromatography. It was found that glycerol also was dissolved in the SC- CO<sub>2</sub>/methanol mixture and separated upon standing in the product mixture after decompression from the SC- CO<sub>2</sub> stream. This probably contributed to the observed high activity of the lipase during continual use throughout the course of these studies. It was found that only a minimal amount of water was required to maintain enzyme activity during the course of these experiments; in fact a water content greater than 0.05 volume % in CO<sub>2</sub> tended to inhibit the methanolysis reaction.

The high pressure glycerolysis reaction was studied using the system shown in Figure 2. Here carbon dioxide was delivered to a 300 mL Magnedrive stirred autoclave from Autoclave Engineers (Erie, Pennsylvania, USA) using a gas booster pump (Model AGT- 162/152, Haskel, Inc., Burbank, California, USA). The reactor was charged with the requisite amounts of reactants (oil, water, and glycerol), equally between 110-115 g, filling approximately 1/3 of the reactor vessel. A CO<sub>2</sub> purge was conducted for 30 min to remove any air at a stirring speed of 180 rpm, and then the reactor was heated to the desired reaction temperature. The reactor was then pressurized and the agitator speed increased to approximately 1000 rpm before starting the reaction. Reactions were usually run for 4 hr and intermittent samples were taken every 30 min to track the course of the reaction. A special precaution were taken to



**Figure 1:** Schematic of the continuous flow system for the fatty acid methyl ester synthesis from seed oils. Legend: (A) CO<sub>2</sub> source, (B,C) CO<sub>2</sub> syringe pumps, (D) methanol pump, (E) oil pump, (F) check valves, (G) enzyme bed in reactor oven, (H) the heated restrictor.



**Figure 2:** Schematic of the stirred autoclave reactor system.

cool the samples rapidly after removal from the autoclave. In addition, the autoclave was also rapidly cooled after completion of the reaction to avoid any reversion of the products.

Analysis of the product mixtures was accomplished on a Dionex Model 602 supercritical fluid chromatograph using a SB-Octyl 50 capillary column. This unit permitted the rapid separation and quantitation of the major reaction products from the glycerolysis, namely mono-, di-, and tri-glycerides plus fatty acids. Quantitation was performed using a docosane internal standard and determining specific response factors for docosane, mono-, di-, and tri-olein. A partial factorial design was also used in optimizing the experiments with respect to the chosen pressures, temperatures, glycerol/water molar ratios, and water content of the reaction. These and other experimental details are given in forthcoming publications [18,19].

### 3. RESULTS AND DISCUSSION

Enzymatic conversion of the seed oils to FAMES was optimized at 17.2 MPa and 50°C, conditions consistent with the maintenance of enzyme activity. This corresponds to an approximate SC- CO<sub>2</sub> density of 0.83 g/mL. Methanol addition was also optimized with respect to the corn oil addition rate at a 25:1 molar ratio of methanol to oil. Higher addition of methanol tended to inhibit the reaction, while a lower molar addition of the alcohol was insufficient for a good reaction yield. Conversions were 98% or greater using the above conditions, however attempts to increase oil solubility in SC- CO<sub>2</sub> [20] for an increased production of FAMES resulted in only a 67% conversion of the triglycerides at 24.1 MPa. This result may be due to the inability of the lipase to convert the three-fold higher concentration level of the oil in the SC- CO<sub>2</sub> at the flow rate utilized in the reaction. The higher pressure may also contribute to a reduction in the lipase activity, resulting in a lower conversion efficiency. Table III shows the FAME product distribution for triplicate conversions of soybean oil, extracted from flakes over the lipase catalyst. The data indicate that reproducible conversions can be achieved using the above described flow systems. Further, the product distribution is equivalent to the FAME composition recorded for "soy diesel", produced by the base-catalyzed methanolysis of oil in a stirred reactor using NaOH.

Fatty Acid	Determination #			AVG	RSD	Soy Diesel
	1	2	3			
Palmitic	12.1	11.3	11.6	11.6	2.83	10.6
Stearic	4.8	5.2	5.2	5.1	3.14	4.5
Oleic	23.9	24.3	23.8	24.0	0.91	23.7
C18:1d11	1.53	1.54	1.51	1.53	0.72	1.29
Linoleic	52.3	52.3	52.3	52.2	0.33	51.1
Linolenic	5.6	5.4	5.6	5.6	2.02	7.1

Table 3: Quantitative determination of soybean oil fatty acid composition from lipase catalysis under supercritical fluid conditions.

The above enzyme catalyzed reaction system has also been used with minor modification, to synthesize FAMES for nutritional labeling analysis of the total fat content of foodstuffs, such as meats. In this case, the micro analytical sample (100 mg) is placed in the reactor cell ahead of the enzyme catalyst bed and the fat is extracted into the SC- CO<sub>2</sub>/methanol fluid mixture before it is directed over the lipase catalyst. The resultant FAMES as noted previously drop out of solution at the restrictor and can be collected in a vial for gas chromatographic analysis. Table 4 shows the results of such an analysis in which total, saturated, and monounsaturated fat have been determined, using in one case the SFE/SFR tandem system described above, and in the other case, conventional solvent extraction of the fat [21] followed by BF<sub>3</sub>-catalyzed transesterification of the fat to FAMES. The quantitative agreement is quite good and within the limit of experimental error, verifying the usefulness of this approach for the analysis of fat in meat products. Recently, this has been automated to allow the extraction-reaction to be conducted in the extraction cell, with subsequent collection of the FAMES after depressurization into a injection vial for gas chromatography, followed by robotic transfer of the sample vial on to the injection tray of the gas chromatographic unit. The use of this enzymatic "hydrolysis" avoids laborious and chemical intensive sample preparation methods, such as acidic hydrolysis followed by organic solvent extraction.

Sample/Extraction	Type of Fat		
	Total	Saturated	Monounsaturated
Bacon/Solvent	38.7 (1.5)*	14.1 (1.5)	18.5 (1.6)
Bacon/SFE-SFR	40.9 (2.8)	14.4 (3.5)	18.4 (2.2)

Mean and Relative Standard Deviation (RSD) = %

**Table 4:** Comparison of SFE/SFR and solvent extraction results for total fat analysis.

The high pressure glycerolysis reaction produces in addition to the desired monoglycerides, intermediate reaction products such as diglycerides, and fatty acids from the competing hydrolysis reaction. A typical conversion shows an exponential decrease in triglyceride concentration as a function of time, while the diglycerides initially increase in concentration and reach a maximum, followed by a decrease in concentration as they are converted to monoglycerides. This is the trend expected for a reaction based on consecutive conversion steps of triglyceride to the monoglyceride moiety. The competing hydrolysis reaction increases the fatty acid content initially with respect to time but then gradually decreases due to the free fatty acid reacting with glycerol to form additional monoglyceride.

A certain optimal temperature (250°C) was required to produce significant quantities of monoglycerides, regardless of the ratio of the reactants, pressure, or water content. The use of reaction temperatures above 250°C is also essential when the glycerolysis is conducted at approximately ambient conditions; primarily to the assure mutual miscibility between the oil and glycerol reaction components. The effect of the other reaction variables, pressure and glycerol/oil ratio are summarized in Figure 3. As expected, there is an increase in monoglyceride formation as the glycerol/oil ratio is increased, although this is suppressed at higher reaction pressures. The large excess of glycerol displaces the equilibrium reaction

toward the right, and requires removal from the product mixture after completion of the reaction. The effect of pressure on the reaction is complex due to the change in solubility of the reactants and products with pressure, however it is well known that pressure can exert a positive effect on the rate of the reaction if the activation volume of the reaction is negative [22]. As noted in Figure 3, an increase in SC-CO<sub>2</sub> pressure appears to inhibit the production of monoglycerides. This may be partially due to the fact that both glycerol and soybean oil exhibit different solubilities in SC-CO<sub>2</sub> as a function of pressure and this can significantly affect the partition of these components between the liquid and dense gas phases. A similar argument has been advanced by Erickson et al. [23] for the observed decrease in conversion rate in a lipase acidolysis between palmitic acid and trilaurin. It should be noted however that the conversion rate to monoglycerides is higher at 20.7 MPa than that achieved from a similar reaction run under subcritical conditions (5.5 MPa), resulting in a 30% increase in the monoglyceride content of the product mixture. This result illustrates the potential benefit of operating under supercritical or high pressure conditions.

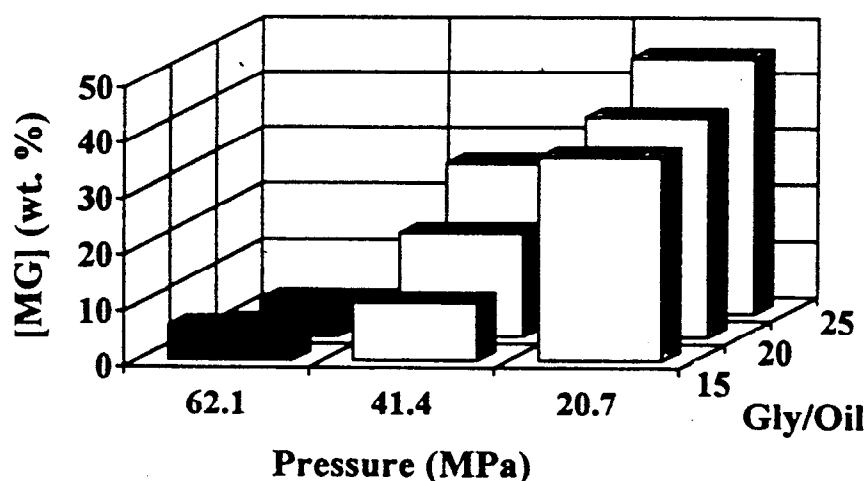


Figure 3: Monoglyceride content in product mixture after 4 hrs. of glycerolysis at 250°C and a 4% water level as a function of pressure and glycerol/oil ratio.

Variation of the water content of the glycerolysis reaction also had an effect on the monoglyceride yield. Monoglyceride content practically doubled when the water content was raised from 0 to 2 %. Glycerolysis conducted under similar conditions, but using different vegetable oils, gave the highest monoglyceride yields with castor oil and the lowest for cottonseed oil. It would appear that some of these conversion differences are related to the solubility of glycerol in the various oil matrices [24].

In summary, it would appear that there are some advantages to conducting glycerolysis reactions in SC-CO<sub>2</sub>. The inclusion of carbon dioxide eliminates the need for conventional alkali-based catalysts and the need to remove these from the product mixture after completion of the reaction (i.e., CO<sub>2</sub> can be removed from the reaction mixture simply by reducing the



pressure). In addition, monoglyceride conversion rates are higher than those obtained under conventional glycerolysis conditions and CO<sub>2</sub> affords a protective atmosphere that reduces the effect of O<sub>2</sub> at the high temperatures (the product has a lighter color and is devoid of odor).

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